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Identification of estrogen-responsive DNA sequences by transient expression experiments in a human breast cancer cell line

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ABSTRACT

The expression of a hybrid gene formed by the promoter region of the Xenopus laevis vitellogenin gene B1 and the CAT coding region is regulated by estrogen when the gene is transfected into hormone-responsive MCF-7 cells. Furthermore, the 5' flanking region of the gene B1 alone can confer inducibility to heterologous promoters, although to a varying extent depending on the promoter used. Deletion mapping of the vitellogenin hormone-responsive sequences revealed that a 13 bp element 5'-AGTCACTGTGACC-3' at position -334 is essential for estrogen inducibility. We have shown previously that this 13 bp element is present upstream of several liver-specific estrogen-inducible genes (1).

INTRODUCTION

Steroid hormones can control gene expression both at the transcriptional and posttranscriptional levels. For the regulation of transcription, it is thought that the hormone-receptor complexes, as eukaryotic transcriptional regulatory factors, interact with specific DNA sequences located close to the initiation site of transcription. Such hormone-responsive DNA elements have been identified for a few hormone-receptor complexes. For instance, the glucocorticosteroid receptor binds at a specific DNA sequence in the promoter region of the genes for chicken lysozyme (2), mouse mammary tumor virus (3,4) rabbit uteroglobin (5), human metallothionein IIA (6) and human growth hormone (7). Similarly, a progesterone receptor binding site has been identified in the chicken lysozyme 5' end gene region (8). Knowledge about hormone-receptor complex binding sites is less advanced in estrogen-regulated gene systems, but suitable genes are now available for analysis. In Xenopus laevis, for instance, the vitellogenin genes are strictly controlled by estrogen in the liver. We have previously isolated the four Xenopus genes and have analyzed their 5' flanking region with the

hope to find common sequence elements that would provide insight to the mechanism of their estrogen-regulated expression. The analysis which included a chicken vitellogenin gene revealed four blocks of homology at equivalent positions in all five genes (1). Within homology block-4, we identified a 13 bp sequence with two-fold rotational symmetry (consensus GGTCANNNTGACC). This element is present in one to three copies upstream of the five analyzed genes as well as in two copies close to the 5' end of the chicken apo-VLDLII gene which is also estrogen-responsive in the liver. Our hypothesis is that the regulation of the vitellogenin genes is mediated by these conserved DNA sequences which might represent binding sites for transcription factors, including the estrogen receptor.

Unfortunately, there is no permanent Xenopus cell lines derived from the liver or, alternatively, Xenopus cell lines that contain estrogen receptors, in which we could analyze the possible functions of these sequences. However, the human breast cancer cell line MCF-7 contains the receptor (9,10). In these cells, the activity of a marker gene, the pS2 gene, which has been shown to be regulated at the transcriptional level by estrogen, is an excellent indicator of the induced state (11). Thus, we have used this system to analyze the sequence requirements for the estrogen-dependent activation of transcription from a Xenopus laevis vitellogenin gene promoter. A test gene, controlled by deletion mutants of the 5' flanking region of the vitellogenin gene B1, was transfected into the MCF-7 cells and analysis of its expression revealed the DNA sequence which is responsible for estrogen responsiveness. Experiments performed by Klein-Hitpass et al. with the related vitellogenin gene A2 gave similar results (12).

MATERIAL AND METHODS

Plasmid constructions and DNA preparation

Plasmids pSV2-CAT (13) and CAT-2 were obtained from P. Gruss (Heidelberg). Plasmid CAT-2 (Fig. 1A) contains the SV-40 early promoter without enhancer sequences (positions 68-5107), the CAT gene coding sequences, the SV-40 small t splicing site (positions 4710-4100) and the SV-40 polyadenylation signals (positions 2771-2534) in a pBR322 derivative vector (13,14). Plasmid pBL-CAT8+(Fig. 1A) was a gift from G.U. Ryffel (Karlsruhe). This plasmid contains the Herpes simplex virus TK promoter (-105/+51) directing

transcription of the CAT gene. As for CAT-2, the pBL-CAT8+ plasmid contains the SV-40 small t splicing site and polydenylation signals. Plasmid pB1(-596/+8)-CAT8+ was generated by inserting a vitellogenin gene B1 fragment (-596/+8) via BamHI linkers into pBL-CAT8+ lacking a BamHI - BglII fragment containing the TK promoter. Both vector/insert junctions were sequenced. Plasmid pBLO-CAT8+ is recircularized pBL-CAT8+ lacking a promoter (Fig. 1A). Plasmids pB1(-596/-42)-SV40-CAT and pB1(-596/-42)TK-CAT8+ were constructed by inserting a BamHI (linker) / BglII B1 vitellogenin fragment (positions -596 to -42) into the BglII site of CAT-2 and into the BamHI site of pBL-CAT8+ (Fig. 1A).

The Exonuclease III method was used to generate the 5' and 3' deletion mutants (15). For the 5' deletion mutants, a vitellogenin gene B1 PstI fragment (-2600 to + 1129) was subcloned into a pUC9 derivative with the following expanded polylinker: EcoRI/XhoI/BamHI/KpnI/BamHI/SalI/PstI/HindIII (D. Nardelli, unpublished). After digestion with SalI and KpnI, the DNA was treated with Exonuclease III and subsequently with S1 nuclease. The vector was recircularized and the endpoint of the deletions was determined by sequencing. The deletion mutants were recovered by a double digestion with BamHI (polylinker site) and BglII (B1 gene position -42) and subcloned into the BamHI site of pBL-CAT8+.

To create the 3' deletion mutants, the same PstI fragment (-2600 to +1129) was introduced into the PstI site of pUC9. A BglII digestion, followed by a PstI partial digestion, allowed us to isolate a fragment containing B1 DNA (-2600 to -42) linked to the vector. A series of 3' deletions was generated as described for the 5' deletion mutants. The fragments of interest were recovered by a double digestion with PvuII (site at position -564 in the gene B1) and BamHI (pUC9). These fragments were introduced into the vector pBL-CAT8+ via filled-in HindIII and BamHI termini.

The plasmids used in transfection experiments were prepared either by the Triton lysis or alkaline lysis methods (16,17). DNA was purified on two CsCl/Ethidium bromide gradients. To completely eliminate the contaminating RNA, the plasmid DNA was further purified either by gel filtration through Biogel A50 or on NaCl gradients.

Cell culture, transient transfection and hormonal induction

MCF-7 cells (9) were routinely grown in Dulbecco's modified Eagle's Medium (DMEM) containing 10% Fetal Calf Serum (FCS), 4 µg/ml insulin and antibiotics. Two days before plating for transfection, the medium was changed to DMEM containing 10% New Born Calf Serum (NBCS). Twenty four hours before transfection, the cells were plated at a density of 0.1 to 0.2 x 10^5 cells/cm^2 in transfection medium: DMEM containing 10% NBCS and 1µM Tamoxifen, an estrogen antagonist. The cells were fed with fresh transfection medium two to four hours before transfection, which was performed by the calcium phosphate coprecipitation technique using 1.5 pmole plasmid DNA per 3 to 5x10^5 cells (18,19). After a glycerol shock, the cells were placed in culture medium containing 1µM Tamoxifen and 10% FCS that had been stripped of endogenous steroids (20), and cultivated for 15 to 20 hours with or without 2x10^{-7} M 17ß-estradiol.

Cell free extracts and CAT assay

15 to 20 hours post-transfection, the cells were scraped from the dishes in ice-cold TNE (40 mM Tris-HCl pH 7.5 / 150 mM NaCl / 1 mM EDTA), washed several times in the same buffer and finally resuspended in 0.25 M Tris-HCl pH 7.8. Lysis was achieved by three cycles of freezing and thawing. After a 15 min spin in a microcentrifuge at 4°C, protein concentration in the supernatants was determined using the Bio-Rad Protein Assay. Identical amounts of protein were then used to determine the relative CAT activity as described (21).

Total cellular RNA extraction and primer extension

Total cellular RNA was prepared using guanidinium HCl (22). Primer extensions were performed as described (23) with several modifications. The concentration of KCl was 250 mM during hybridization and 83 mM during elongation, and Murine Moloney Leukemia Virus Reverse Transcriptase (Mu-MoLV RTase, BRL) was used at a concentration of 5000 U/ml. The primer used for mapping the transcription initiation sites of transcripts from the CAT gene was 3'-TACCTCTTTTTTTAGTGACCTATATGGTGG-5'. This primer is complementary to the first 30 coding nucleotides of CAT (24). The oligonucleotide used to map the 5'end of the alpha-2 globin transcripts was 3'-CCCATTCCAGCCGCGCGT GCGACCG-5'; it is complementary to positions +80 to +104 of the alpha-2

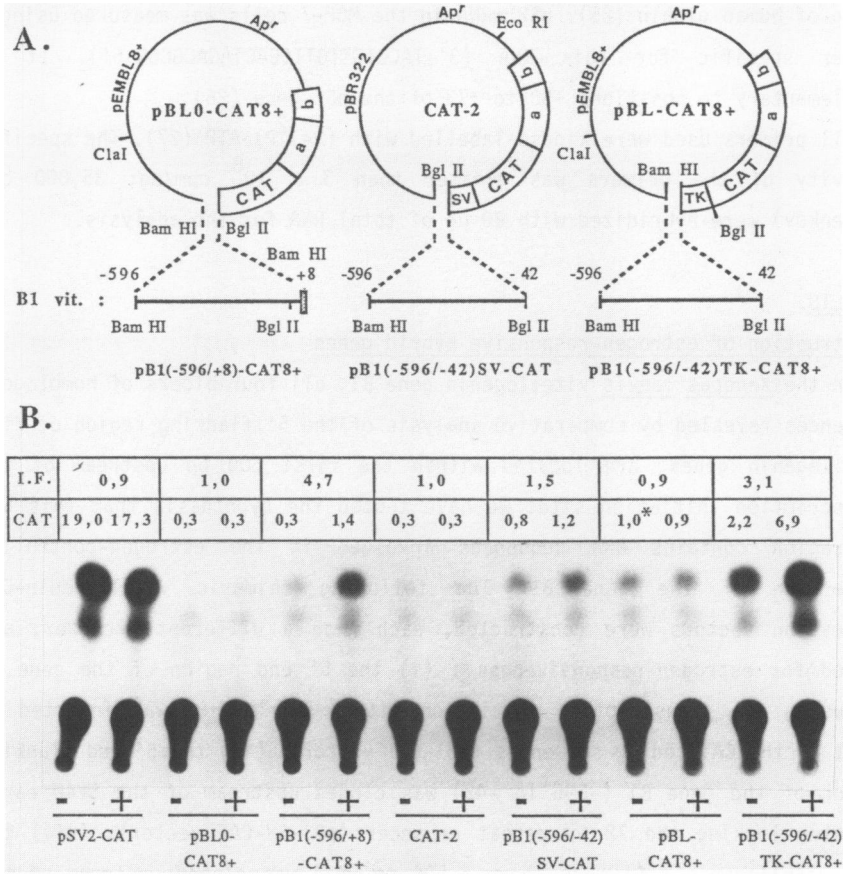


Figure 1 Structural features and expression of vitellogenin-CAT vectors.

A) Structure of the vectors. Thin lines represent vector sequences. Open boxes stand for : (CAT), chloramphenicol acetyl transferase gene; (a), SV-40 small t splicing sequences; (b) SV-40 polyadenylation signals; (SV), SV-40 early promoter without the 72 bp repeat, positions 68 to 5107; (TK), Herpes simplex virus thymidine kinase promoter, positions -105 to +51. Bold lines represent 5'-end proximal fragments of the Xenopus laevis vitellogenin gene B1 whose extremities are defined by their position on the sequence. The dotted box on the right of the -596 to +8 fragment represents the sequence from the cap site (+1) to the nucleotide +8.

B) CAT activities in extracts of MCF-7 cells transfected with the genetic constructions shown in (A) and cultured in the absence (-) or presence (+) of 2×10^{-7} M 17β-estradiol. Each of the aliquots contained 40 µg protein. IF is the induction factor calculated from the mean CAT activity (CAT) determined from four independent experiments. The CAT activities in the extracts of unstimulated cells transfected with pBL-CAT 8+ were taken as one (1*). One of the four experiments is shown.

chain of human globin (25). pS2 mRNA in the MCF-7 cells was measured using a primer specific for this RNA (3'-TACCTCTTGTTCCACTAGACGCGG-5'). It is complementary to positions +50 to +73 of the pS2 gene (26).

All primers used were kinase-labelled with (γ-^{32}P)-ATP (27). The specific activity of the primers was greater than 3 x 10^8 cpm/µg; 35,000 cpm (Cerenkov) were hybridized with 20 µg of total RNA for the analysis.

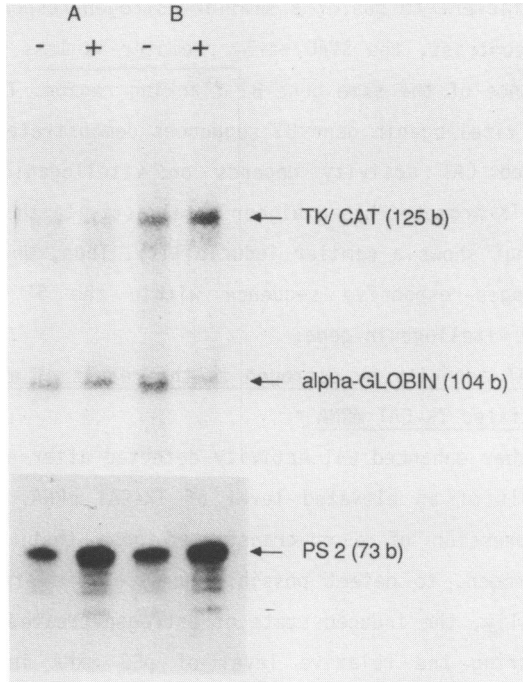
RESULTS

Construction of estrogen-responsive hybrid genes

In the Xenopus laevis vitellogenin gene B1, all four blocks of homologous sequences revealed by comparative analysis of the 5' flanking region of five vitellogenin genes, are located within the first 600 bp upstream of the transcription initiation site. We have tested the hypothesis that this 600 bp region contains DNA sequences involved in the estrogen-controlled expression of the gene B1. The following chimeric vitellogenin-CAT expression vectors were constructed, each with a different promoter, and tested for estrogen-responsiveness : (i) the 5' end region of the gene B1 including the transcription initiation site (-596 to +8) was inserted in front of the CAT coding sequences (pB1-CAT vector); (ii) the 5' end flanking region of the gene B1 (-596 to -42) was cloned upstream of the SV40 early promoter lacking the 72 bp repeat enhancer (pB1-SV-CAT vector); (iii) the same vitellogenin region as above (-596 to -42) was cloned upstream of the Herpes simplex virus TK promoter (pB1-TK-CAT8+ vector).

These three constructions and their corresponding parental plasmids (Fig. 1A), as well as the pSV2-CAT plasmid as positive control, were transfected independently into MCF-7 cells. The cells were then stimulated with 17ß-estradiol or, as a control, left unstimulated.

After 15 to 20 hours, crude cell extracts were prepared and the level of CAT activity in the extracts was measured. Figure 1B gives the results. The vitellogenin gene B1 promoter pB1(-596/+8) is weak in unstimulated MCF-7 cells. Upon estrogen stimulation, however, a substantial rise in CAT activity is observed demonstrating that the promoter responds to hormonal induction. Furthermore, the 5' flanking region of the gene B1 by itself



	GLOBIN	CAT	induction	PS2	induction
A-	71	45		184	
A+	76	45	1.0	427	2.2
B-	54	61		246	
B+	50	163	2.9	448	2.0

Figure 2 Analysis by primer extension of CAT, α-globin and pS2 transcripts in transfected MCF-7 cells. The autoradiograms represent data from primer extension with 10 µg total RNA prepared from MCF-7 cells transfected with the following DNAs : (A) equimolar mixture of pBL-CAT8+ and pUC9-α-globin, (B) equimolar mixture of pB1(-344/-42)TK-CAT8+ and pUC-α-globin. The cells were either unstimulated (-) or treated with 17β-estradiol (+). The RNAs were hybridized either to a mixture of end-labelled CAT and globin alpha-2 primers or, alternatively, pS2 primer, and the primers were extended with reverse transcriptase. The position of the correct start site for each transcription unit is indicated by an arrow and the length of the extended products is given in brackets. Quantification of the relative amount of transcripts in each band was performed by densitometric scanning of the autoradiogram. The values obtained are given in arbitrary units. The values for estradiol induction of the CAT and pS2 transcripts have been standardized to the amount of α-globin trancripts.

(-596/-42) is sufficient to confer a similar estrogen-responsiveness to the TK promoter. In contrast, the SV40 early promoter is less sensitive to the regulatory influence of the same gene B1 flanking region. The expression of plasmids lacking vitellogenin gene B1 sequences demonstrates that estrogen-dependent enhanced CAT activity depends on vitellogenin sequences. It appears that the TK promoter has a higher basal activity than the vitellogenin promoter B1 but shows a similar inducibility. Thus, we have used it to search the estrogen-responsive sequence within the 5' flanking region (-596/-42) of the vitellogenin gene.

Stimulation of CAT activity by estrogen is the result of an elevated level of correctly initiated TK-CAT mRNA

We tested whether enhanced CAT activity detected after estrogen stimulation is the result of an elevated level of TK-CAT mRNA. In addition, we analyzed the expression of a co-transfected gene that is normally not regulated by estrogen, to detect possible non-specific effects of hormone stimulation. Finally, the induced state of estrogen-treated MCF-7 cells was checked by measuring the relative level of pS2 mRNA in stimulated and unstimulated cells.

In practice, an estrogen inducible pB1-TK-CAT plasmid or, alternatively the parental pBL-CAT8+ plasmid lacking vitellogenin sequences, was co-transfected with a cloned human alpha-2 globin gene. The levels of TK-CAT mRNA, human alpha-2 globin mRNA and pS2 mRNA were measured by elongation of synthetic primers hybridized to total cellular RNA from the transfected, stimulated or unstimulated cells (Fig. 2). From the results obtained, it is clear that the initiation of transcription of all the transfected genes is faithful. Furthermore, the CAT gene driven by the TK promoter alone is not influenced by estrogen treatment of the cells. However, the 2.2-fold increase in pS2 transcripts shows that the cells were induced correctly (Fig. 2A). In contrast, the cells transfected with the estrogen-inducible pB1-TK-CAT plasmid show a 3-fold higher level of TK-CAT mRNA when stimulated, compared to the globin mRNA level (Fig. 2B). The level of pS2 mRNA is again increased 2-fold after hormonal treatment. Thus, these results demonstrate that the elevated CAT activity, found only in MCF-7 cells

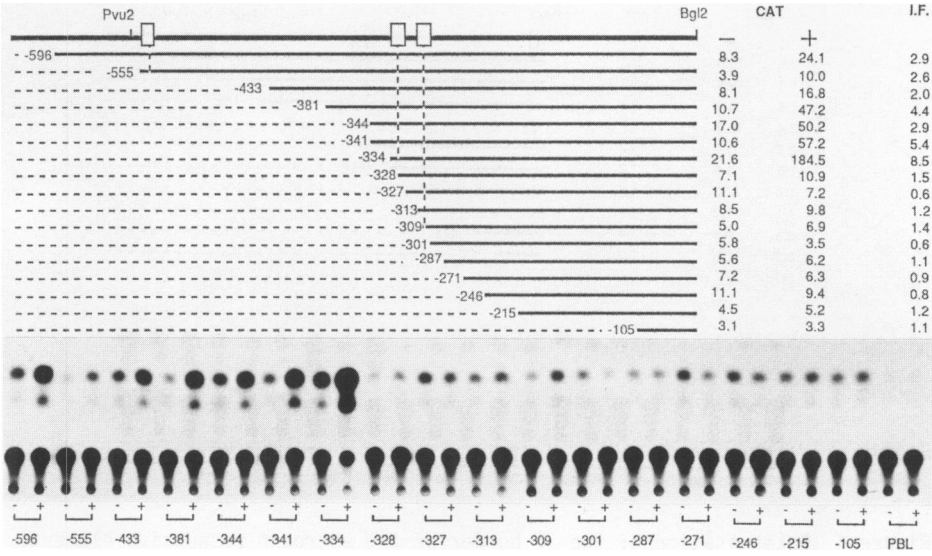


Figure 3 Determination of the 5' border of the estrogen-responsive DNA sequences. (A) Scheme representing the 5' deletion mutants pB1 (Δ 5'/-42)TK-CAT8+. Numbers at the left side give the position in bp of the 5' terminal nucleotide of the Xenopus DNA fragment relative to the transcription initiation site of the gene B1. The open boxes represent the three 13 bp palindromic elements found in the gene B1. CAT is the mean CAT activity, calculated from three independent experiments, in extracts derived from control (-) and estrogen-treated (+) cells. IF is the induction factor as described in the legend of Figure 1.

(B) Example of a CAT assay performed with extracts (20 µg protein) of MCF-7 cells transfected with the mutants diagrammed in (A). Numbers at the bottom correspond to the end points of the 5' deletion mutants as in (A). (-) and (+) refer to control and estrogen-treated cells, respectively.

transfected with pB1-TK-CAT plasmids and treated with estrogen, is the consequence of an increased level of specific mRNA molecules.

Localization of the 5' end border of the estrogen-responsive DNA sequences

Clearly, the DNA fragment flanking the gene B1 (-596/-42) can confer estrogen-responsiveness to the TK-CAT hybrid gene (Fig. 1 and 2). To identify the region responsible for this effect, the gene B1 DNA fragment was progressively truncated at its 5' end. The inducibility of the resulting 5' deletion clones was tested to determine the 5' border of the estrogen-

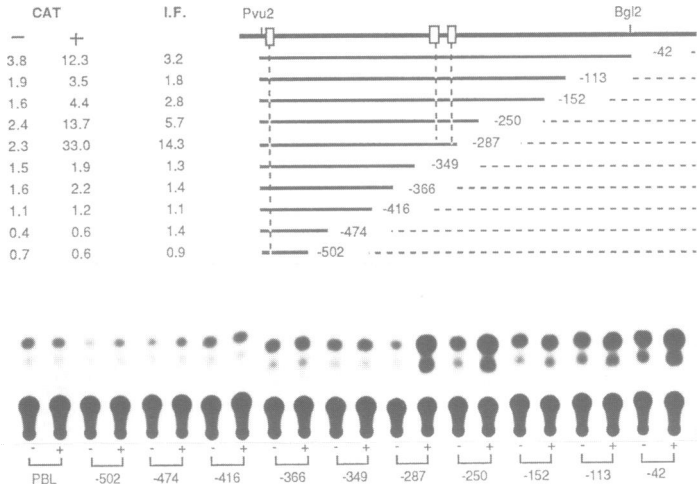


Figure 4 Determination of the 3' border of the estrogen responsive element.
(A) Diagram of the 3' deletion mutants : pB1(-564/Δ 3')TK-CAT8+. Numbers at the right side are the end points of the 3' deletions in bp. They indicate the position relative to the transcription initiation site of the gene B1. At the left are shown the CAT activities for each mutant and the induction factors determined as described in Figure 1.
(B) Example of a CAT assay performed with extracts (40 µg protein) of MCF-7 cells transfected with the recombinants diagrammed in (A). Numbers at the botton correspond to the endpoints of the 3' deletions as in (A). (-) and (+) are for control and estrogen-stimulated cells, respectively. PBL refers to the control vector pBL-CAT8+ without any gene B1 vitellogenin insert (see Figure 1).

responsive DNA sequences (Fig. 3). Deletions down to position -334 still are estrogen-responsive while deletions to position -328 and further downstream have lost the hormone sensitivity. As the deletions approach the point where responsiveness is abruptly lost, the estrogen-inducibility gradually increases to reach a factor of about 9 (Fig. 3).

The 5' end region of the vitellogenin gene B1 contains three copies of the 13 bp palindromic element mentioned in the introduction at positions -555, -334 and -314 (position of the 5'end nucleotide of the element). Deletion of the upstream copy of this element does not abolish estrogen sensitivity, while partial or full deletion of the central copy (-334 element) results in loss of inducibility, demonstrating that the -314 copy alone is not sufficient to confer hormone responsiveness.

Determination of the 3' border of the estrogen-responsive DNA sequences

A gene B1 DNA fragment (-564/-42) equivalent to the one used to prepare 5' deletions was truncated from its 3' end starting at position -42. These 3' deletion fragments were fused to the TK-CAT gene used above and tested for estrogen responsiveness after transfer into control or stimulated MCF-7 cells (Fig. 4). The ability to respond to the hormone is lost between positions -287 and -349. The region between these positions contains the -334 and -314 copies of the 13 bp palindromic element. As observed with the 5' deletions, when the 3' deletions near the point where responsiveness is lost, the inducibility is increasing up to a factor of about 14 (Fig. 4). Finally, the upstream -555 copy of the 13 bp palindromic sequence, together with its flanking sequences, does not confer significant estrogen inducibility.

Characteristics of the estrogen-responsive DNA sequences

A composite representation of the 5' and 3' deletion mutant experiments is given in Figure 5 for clarity. Together these experiments define a region from position -334 to -287 which contains the estrogen-responsive DNA sequences. It appears that when relatively long Xenopus DNA sequences are flanking this region on both sides, its ability to mediate the hormone action is reduced. The region whose primary sequence is shown in Figure 5 contains the -334 and -314 copies of the 13 bp element and also a large palindrome that partially overlaps the upstream 13 bp element. The integrity of the large palindrome is not a prerequisite for estrogen-responsiveness since the 5' border of the estrogen-responsive sequences coincides with the 5' end of the -334 copy of the 13 bp element. Further 3' deletions will be necessary to determine if the adjacent -314 downstream element, which is non-responsive by itself, is participating in the hormonal induction.

DISCUSSION

In oviparous vertebrates, the activity of the vitellogenin genes is strictly regulated by estrogen in the liver of vitellogenic females. Although permanently dormant in males, these genes can be induced by a single estrogen injection and their activation in hepatocytes is independent of further cytodifferentiation or cell proliferation (28,29). To identify

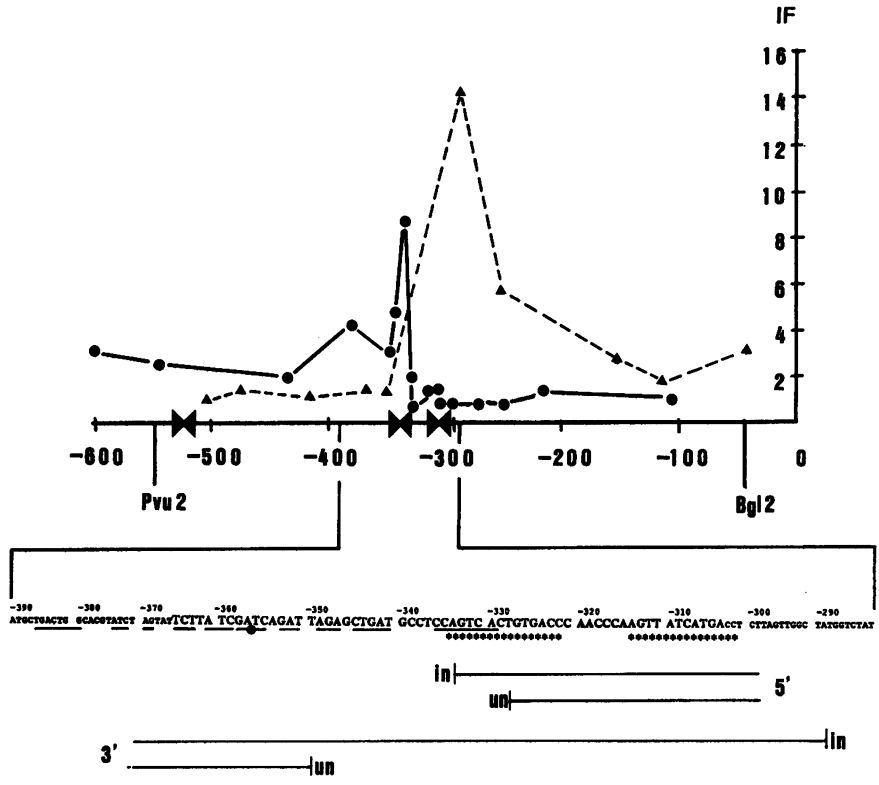


Figure 5 Localization of the estrogen-responsive DNA sequences in the Xenopus vitellogenin B1 gene. Diagram showing the induction factor (I.F., see Fig. 3 and 4) conferred by each of the 5' deletion mutants (●) and 3' deletion mutants (▲) as a function of the endpoint of the deletions relative to the gene B1 initiation site of transcription. The positions of the 13 bp elements are indicated on the X-axis (⋈). The gene B1 nucleotide sequence from -281 to -390 is shown. The -334 and -314 copies of the 13 bp element are identified by stars. The sequence forming a large palindrome whose center is indicated by a dot is underlined. Nucleotides of the homology block-4 as defined earlier (ref. 1), are presented with bigger characters. The endpoints of the last inducible (in) and first uninducible (un) 3' and 5' deletions mutants are given below the sequence.

the DNA sequences which confer estrogen-inducibility to these genes we have transfected the estrogen-responsive human breast carcinoma cell line MCF-7 with hybrid genes whose expression is under control of the 5' end region of the Xenopus vitellogenin gene B1.

The results obtained demonstrate that vitellogenin 5' flanking sequences

contain a DNA region which confers estrogen-responsiveness to the vitellogenin or other promoters when tested in the MCF-7 cells. Thus, it appears that the hormonal inducibility of the vitellogenin gene B1 is mediated by DNA sequences located in the 5' flanking region of the gene. This does not exclude the possible involvement of other regions, which have yet to be identified, in the regulated expression of the vitellogenin genes in hepatocytes. Obviously, a human cell line is an unnatural host for amphibian DNA sequences. However, we have shown previously that factors of the human transcription machinery can interact correctly with vitellogenin gene promoters (30, 31, 32).

It is thought that the estrogen receptor, which most likely is involved in the induction effect we have observed here, functions as a transcriptional regulatory factor. Recently, the analysis of the amino acid sequence of the human and chicken estrogen receptor revealed high homology in three regions two of which corresponding to the putative DNA and estradiol binding domains (33). Most likely, homology also exists with the Xenopus receptor whose amino acid sequence is not yet known. The high interspecies homologies described may explain the interaction of human regulatory factors most likely including the estrogen receptor, with the amphibian vitellogenin gene B1 in MCF-7 cells. Similarly, it explains the regulated expression of the estrogen-sensitive chicken ovalbumin gene that has been observed when the gene was transfected into the same cell line. For this gene, however, the responsive sequences have not been identified (34). Finally, the chicken lysozyme gene has also been transfected into MCF-7 cells but was not found to be regulated by estrogen (35). Thus, the estrogen-responsive breast carcinoma cell line is a convenient host to study steroid regulation of some responsive genes but, obviously, the associated tissue specific controls will have to be analyzed in the differentiated cells that normally express the genes.

Clearly, our results show that the level of induction conferred by the vitellogenin 5' flanking region depends upon the promoter utilized. The Herpes virus TK promoter responds similarly as the proper vitellogenin promoter, although it has a higher basal activity in the MCF-7 cells. In contrast, the SV-40 early promoter without its 72 bp repeat enhancer is

significantly less influenced by the vitellogenin estrogen responsive DNA sequences. Most likely, this is the result of the different functional organization of these promoters leading to different combinations of factors affecting transcription.

Functional analysis of the 5' end region of the gene B1 reveals that the 13 bp sequence element described previously is involved in the estrogen-regulated expression of the gene. The gene B1 flanking region presents three copies of the element, one at position -555 and two in the estrogen-responsive homology block-4 at positions -334 and -314. Of these two 13 bp elements, the -334 copy, is essential. If it is partially deleted at its 5' end, the remaining -314 copy of the element is inefficient in mediating the hormonal effect. Similarly, the -555 upstream element seems not to have an important role in the induction observed in our experiments. It remains to be elucidated what differentiates the strong -334 element from the two others. In addition, further 3' deletions will be necessary to determine if the -334 element by itself is sufficient to confer estrogen-responsiveness, since it has only been tested associated either with the -555 or the -314 element.

Results from a similar analysis with the gene A2 that has a single 13 bp element at position -331 have also shown that this element is involved in estrogen-responsiveness (12). In addition, further support to our results comes from binding studies with the chicken vitellogenin gene suggesting that the 13 bp palindromic element participates in estrogen receptor binding. The binding site reported comprises the 3' half and downstream sequences of a copy of the 13 bp element (36). In agreement with this observation preliminary experiments (not shown) reveal that the 13 nucleotide palindromic element by itself is not sufficient to mediate estrogen-response. Since deletions to its 5' border do not abolish estrogen responsiveness, it can be concluded that it needs additional nucleotides in 3' to be functional.

Sequences adjacent to the estrogen-responsive region reduce estrogen sensitivity since their gradual deletion leads to an enhanced hormone inducibility (Fig. 5, see also ref. 12 for the gene A2). Thus, it seems that there is an active negative interference of the deleted sequences. However,

a position effect cannot be excluded with the 3' deletions which bring the estrogen-sensitive sequences nearer to the transcription initiation site. In contrast, this argument does not hold for the 5' deletions. There appears to be some analogy between the functional organization of the gene B1 flanking region and that of the ß-interferon gene (37,38). For the latter, it has been demonstrated that the enhancer which binds the ITF transcription factor is flanked on both sides by two domains interacting with negative regulatory factors in unstimulated cells. The DNA sequence of one of these flanking domains presents also an interrupted dyad symmetry.

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